Amendments to the Claims

Please amend Claims 23, 25-27, 59, 62 and 67. The Claim Listing below will replace all prior versions of the claims in the application:

Claim Listing

1 - 22. (Canceled)

23. (Currently Amended) A method for identifying genes that carry one or more alleles that confer an increased likelihood of mortal disease, comprising

a) determining the number of two or more detectable point mutations in a gene or gene segment in a population of young individuals, and calculating the sum of the frequencies of all point mutations determined for each gene or segment;

b) determining the number of two or more ~~a plurality of~~ detectable point mutations in a gene or gene segment in a population of aged individuals and calculating the sum of the frequencies of the point mutations determined for each gene or segment;

c) comparing the sum of the frequencies of point mutations that are found in a selected gene or portion thereof of the young population calculated in a) with the sum of the frequencies of point mutations that are found in the same gene or portion thereof of the aged population calculated in b),

wherein a statistically significant decrease in the sum of the frequencies of point mutations associated with a particular gene in the aged population indicates that said selected gene carries one or more alleles that confer an increased likelihood of mortal disease.

24. (Canceled)

25. (Currently Amended) A method for identifying genes that carry one or more alleles with an increased likelihood of being harmful ~~a harmful allele~~, comprising:

a) identifying the set of point mutations that are found in one or more genes or portions thereof of a population of young individuals, wherein the set comprises all detectable point mutations occurring at a frequency at about or above 5×10^{-5} in a population from which the sample was obtained, and determining the frequency with which each point mutation occurs;

b) identifying the detectable set of point mutations that are found in the genes or portions thereof of a population of aged individuals, and determining the frequency with which each point mutation occurs; and

c) comparing the frequency of each point mutation identified in a selected gene or portion thereof of the young population determined in a) with the frequency of the same point mutations identified in said selected gene of the aged population determined in b),

wherein a significant decrease in the frequency of two or more point mutations in said selected gene of the aged population relative to said selected gene of the young population indicates that said selected gene carries one or more alleles with an increased likelihood of being harmful ~~a harmful allele~~.

26. (Currently Amended) The method of Claim 25 further comprising:

d) determining the frequency of a subset of the mutations identified in c) that have ~~are~~ significantly decreased frequencies in the aged population, wherein the subset of point mutations are obligatory knockout point mutations;

e) determining the age-specific decrease in frequency of any of said subset of point mutations; and

f) comparing the age-specific decrease in frequency with the expected age-specific decrease in frequency of a set of harmful alleles that cause ~~of~~ a particular mortal disease,

wherein a determination that the age-specific decrease in frequency determined in e) is not significantly different from the expected age-specific decrease in frequency of

harmful alleles further indicates that said selected gene carries one or more alleles with an increased likelihood of being harmful ~~a harmful allele and has a high probability of being causal of said mortal disease~~.

27. (Currently Amended) The method of Claim 25 further comprising:

d) identifying a subset of obligatory knockout point mutations in the point mutations identified in c) ~~as being~~ that have decreased frequencies in the aged population;

e) determining the frequency of the point mutations identified in d), that are significantly decreased in one or more proband populations, wherein the proband population consists of persons diagnosed with ~~the~~ a mortal disease drawn from a common population; and

e) comparing the frequencies of the subset of point mutations in said selected gene or portion thereof in the young population with the frequencies of said point mutations in said selected gene or portion thereof in the one or more proband populations,

wherein a significant increase in the frequencies of said subset of point mutations in the proband population relative to the young population indicates that said gene carries one or more ~~harmful~~ alleles that confer an increased likelihood of ~~risk for~~ said disease.

28. (Previously presented) The method of Claim 27, wherein the proband population consists of individuals with early onset disease.

29 - 58. (Canceled)

59. (Currently Amended) A method for identifying total detectable obligatory knockout point mutations in any target region of a genome of a population, wherein said point mutations increase the likelihood ~~cause or accelerate the appearance~~ of a mortal disease or increase the likelihood of delaying or preventing ~~prevent or delay~~ the appearance of a mortal disease, comprising:

a) separately determining the frequencies of all detectable mutations occurring at a frequency at or above 5×10^{-5} in members of a population that comprises subpopulations selected from the group consisting of young, aged, intermediate age, afflicted with disease, afflicted with a disease of early age onset and afflicted with a disease of late age onset; and

b) determining the frequencies of each mutation of a) within one or more subpopulations,

wherein a decrease in the frequency in the aged population compared to the young population is indicative of an allele that increases the likelihood of ~~causes or accelerates~~ a mortal disease, and an increase in frequency in the intermediate or aged population compared to the young population is indicative of an allele that increases the likelihood of preventing or delaying ~~prevents or delays~~ the appearance of a mortal disease.

60. (Previously presented) The method of Claim 25, wherein said point mutations are identified using a method that comprises:

a) providing a first pool of DNA fragments comprising a gene or portion thereof, wherein said pool is isolated from a population and contains DNA pooled from 10 to 10,000 individuals;

b) amplifying a target region of said gene or portion from each of said fragments in a high fidelity polymerase chain reaction (PCR) under conditions suitable to produce double-stranded DNA products that contains, or may be subsequently attached to, a terminal high temperature isomelting domain that is labeled with a detectable label, and wherein the mutant fraction of each PCR-induced mutation among the PCR products is not greater than about 5×10^{-5} for a pool created by DNA from 10,000 persons;

c) melting and reannealing the product of b) under conditions suitable to form duplexed DNA, thereby producing a mixture of wild-type homoduplexes and heteroduplexes that contain point mutations;

d) separating the heteroduplexes from the homoduplexes based upon the differential melting temperatures of said heteroduplexes and said homoduplexes and

recovering the heteroduplexes, thereby producing a second pool of DNA that is enriched in target regions containing point mutations;

e) amplifying said second pool by high fidelity PCR under conditions where only homoduplexed double-stranded DNA is produced, thereby producing a mixture of homoduplexed DNA containing wild-type target region and homoduplexed DNAs that contain target regions that include point mutations;

f) resolving from step e) hetero-duplexed DNAs containing target regions that include point mutations based upon the differential melting temperatures of the DNAs, and recovering the resolved DNAs that contain a target region that includes point mutations; and

g) determining the sequence of the target region of the recovered DNAs to identify point mutations within the target region.

61. (Canceled)

62. (Currently Amended) A method for identifying genes that carry one or more ~~harmful~~ alleles with an increased likelihood of being harmful, comprising:

a) identifying in one or more samples two or more point mutations that are found in one or more genes or portions thereof of a population of young individuals, determining the frequency with which each point mutation occurs, and calculating the sum of the frequencies of all point mutations identified for each gene or segment;

b) identifying in one or more samples two or more point mutations that are found in one or more genes or portions thereof of a population of aged individuals, determining the frequency with which each point mutation occurs, and calculating the sum of the frequencies of all point mutations identified for each gene or segment;

c) determining the subset of point mutations that are obligatory knock-out point mutations for steps a) and b); and

d) comparing the sum of the frequencies of obligatory knock-out mutations that are found in a selected gene or portion thereof of the young population with the sum of the frequencies of obligatory knock-out point mutations that are found in the same gene or portion thereof of the aged population,

wherein a significant decrease in the sum of the frequencies of obligatory knock-out point mutations in the aged population indicates that said selected gene carries one or more ~~harmful~~ alleles with an increased likelihood of being harmful.

63. through 65. (Canceled)

66. (Previously presented) A method for identifying genes that carry one or more alleles that confer an increased likelihood of mortal disease, comprising:

a) determining the number and nucleotide sequence identities of all detectable point mutations in a gene or gene segment in a population of young individuals, wherein said gene segment consists of the exons and splice sites of genes that code for proteins, wherein detectable point mutations are selected from the group consisting of: point mutations resulting in amino acid substitutions, small insertions or deletions, termination codons or splice site mutations, in a population of young individuals, and calculating the sum of frequencies of all point mutations determined for each gene or segment;

b) determining the number and nucleotide sequence identities of all detectable point mutations in a gene or gene segment in a population of aged individuals, wherein said gene segment consists of the exons and splice sites of genes that code for proteins, wherein detectable point mutations are selected from the group consisting of: point mutations resulting in amino acid substitutions, small insertions or deletions, termination codons or splice site mutations, and calculating the sum of the frequencies of all point mutations determined for each gene or segment; and

c) comparing the sum of the frequencies of the specified type or types of point mutations that are found in a selected gene or segment thereof of the young population calculated in a) with the sum of the frequencies of the specified type or types of point mutations that are found in the same gene or segment thereof of the aged population calculated in b),

wherein a statistically significant decrease in the sum of the frequencies of the specified type or types of point mutations associated with a particular gene in the aged population indicates that said selected gene carries one or more alleles that confer an increased likelihood of mortal disease.

67. (Currently amended) A method for identifying genes that carry one or more alleles that confer an increased likelihood of mortal disease, comprising

a) determining the number and nucleotide sequence identities of all detectable point mutations in a gene or gene segment in a population of young individuals, wherein the gene segment consists of the exons and splice sites of genes that code for proteins and enumerating the detectable point mutations that are expected to result in a nonfunctional gene product, wherein the point mutations are selected from the group consisting of: ~~such as with~~ small insertions or deletions, termination codons, ~~or~~ splice site mutations, gene-inactivating mutations and ~~or~~ any combination thereof, and calculating the sum of the frequencies of all such specified type or types of point mutations determined for each gene or segment;

b) determining the number and nucleotide sequence identities of all detectable point mutations in a gene or gene segment in a population of aged individuals, wherein the gene segment consists of the exons and splice sites of genes that code for proteins and enumerating the detectable point mutations that are expected to result in a nonfunctional gene product, wherein the point mutations are selected from the group consisting of: ~~such as with~~ small insertions or deletions, termination codons ~~and~~ splice site mutations, gene-inactivating mutations and any combination thereof, and calculating the sum of the frequencies of all such specified type or types of point mutations determined for each gene or segment;

c) comparing the sum of the frequencies of specified types of point mutations that are found in a selected gene or segment thereof of the young population calculated in a) with the sum of the frequencies of specified type or types of point mutations that are found in the same gene or segment thereof of the aged population calculated in b),

wherein a statistically significant decrease in the sum of the frequencies of the specified type or types of point mutations associated with a particular gene in the aged population indicates that said selected gene carries one or more alleles that confer an increased likelihood ~~risk~~ of mortal disease.

68. (Previously presented) The method of Claim 25 wherein said point mutations are identified by amplifying DNA obtained from the individual members of the population samples and determining the nucleotide sequence of the DNA.